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10/601,084	06/20/2003	Paul Jan, J. Hooykaas	2183-6028US	6901

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ART UNIT	**PAPER NUMBER**
1636	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/601,084	HOOYKAAS ET AL.	
	Examiner	Art Unit	
	Jennifer Dunston	1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *24 May 2007*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-6,8-21,23 and 24* is/are pending in the application.

4a) Of the above claim(s) *3,6,8,9,13-15 and 23* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,2,4,5,10-12,16-21 and 24* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *20 June 2003* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *1/25/2007*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

This action is in response to the amendment, filed 5/24/2007, in which claims 7 and 22 were canceled, claims 1 and 10 were amended, and claim 24 was newly added. Currently, claims 1-6, 8-21 and 23-24 are pending.

Applicant's arguments have been thoroughly reviewed, but are not persuasive for the reasons that follow. Any rejections and objections not reiterated in this action have been withdrawn. **This action is FINAL.**

Election/Restrictions

Applicant elected "providing a mutant of a component involved in nonhomologous recombination" as the species of method step, "ku70" as the species of component, and "fungus" as the species of eukaryote without traverse in the reply filed on 7/27/2006.

Claims 3, 6, 8, 9, 13-15 and 23 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected species, there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on 7/27/2006.

Currently, claims 1, 2, 4, 5, 10-12, 16-21 and 24 are under consideration.

Priority

Acknowledgment is made of applicant's claim for foreign priority under 35 U.S.C. 119(a)-(d). Receipt of the certified copy of the foreign priority document, EPO 00204693.6, is acknowledged. These papers have been placed of record in the file.

Information Disclosure Statement

Receipt of an information disclosure statement, filed on 1/25/2007, is acknowledged. The signed and initialed PTO 1449 has been mailed with this action.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1, 2, 4, 5, 10-12 and 16-21 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for steering an integration pathway towards homologous recombination in a eukaryotic cell *in vitro* by providing a mutant component of nonhomologous recombination, does not reasonably provide enablement to steer an integration pathway towards homologous recombination in a eukaryotic cell *in vivo* and does not provide enablement for transient inhibition of integration via nonhomologous recombination by providing a mutant component involved in nonhomologous recombination. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims. This rejection was made in the Office action mailed 10/20/2006 and is reiterated below.

Enablement is considered in view of the Wands factors (MPEP 2164.01(A)). These include: nature of the invention, breadth of the claims, guidance of the specification, the existence of working examples, state of the art, predictability of the art and the amount of experimentation necessary. All of the Wands factors have been considered with regard to the

instant claims, with the most relevant factors discussed below.

Nature of the invention: The claims are drawn to a method of directing integration of a nucleic acid of interest to a predetermined site, wherein said nucleic acid has homology at or around said predetermined site, in a eukaryote with a preference for nonhomologous recombination. The preamble of independent claim 1 reads on the integration of a nucleic acid of interest into a eukaryotic cell *in vitro* or *in vivo* (i.e. gene therapy).

The nature of the subject matter is complex, because the nucleic acid must be delivered at a level sufficient to produce a therapeutic outcome (see the discussion below).

Breadth of the claims: The claims encompass the use of the claimed method in any eukaryotic cell of any organism. Further, the claims are drawn to the integration of any nucleic acid at any site in the genome. The complex nature of the subject matter of this invention is greatly exacerbated by the breadth of the claims.

Guidance of the specification and existence of working examples: The specification envisions the use of the claimed method to deliver a gene to a eukaryotic cell *in vivo* (e.g. page 6). The specification envisions replacing a defective p53 with an intact p53 such that the tumoricidal gene is delivered to a predetermined site only in proliferating cells or tumor cells (e.g. page 6; paragraph bridging pages 11-12). Further, the specification envisions the delivery of a therapeutic proteinaceous substance by integration of a nucleic acid into a eukaryotic cell (i.e. gene therapy) (e.g. paragraph bridging pages 11-12). Thus, the specification envisions the treatment of cancer and genetic diseases such as diseases that result from the expression of a defective protein product.

The specification provides little or no guidance with regard to gene therapy applications.

All of the working examples of the specification are directed to the integration of DNA by homologous or nonhomologous recombination in *Saccharomyces cerevisiae* comprising stable mutations in components involved in nonhomologous recombination. The stable cell lines do not allow transient inhibition of nonhomologous recombination.

State of the art: An analysis of the prior art as of the effective filing date of the present application shows the complete lack of documented success for any treatment based on gene therapy. In a review on the current status of gene therapy, both Verma et al (Nature, Vol. 389, pages 239-242, 1997, cited in a prior action; e.g. page 239, paragraph 1) and Palù et al (J. Biotechnol. Vol. 68, pages 1-13, 1999, cited in a prior action; e.g. Abstract) state that despite hundreds of clinical trials underway, no successful outcome has been achieved. The continued, major obstacles to successful gene therapy are gene delivery and sustained expression of the gene. Regarding non-viral methods for gene delivery, Verma et al (1997) indicate that most approaches suffer from poor efficiency and transient expression of the gene (e.g. page 239, right column, paragraph 2). Likewise, Luo et al (Nature Biotechnology, Vol. 18, pages 33-37, 2000, cited in a prior action) indicate that non-viral synthetic delivery systems are very inefficient (e.g. Abstract; page 33, left column, paragraphs 1 and 2). The post filing art indicates that still suffer from inefficient gene transfer (Verma and Weitzman, Gene Therapy: Twenty-first century medicine. Annual Review of Biochemistry, Vol. 74, pages 711-738, 2005, cited in a prior action; e.g. page712, last paragraph). Regarding viral methods for gene delivery *in vivo*, Verma et al (1997), indicate that lentiviral, adenoviral and AAV vectors are capable of delivery genes, but there is a possibility for insertional mutagenesis or toxicity due to an inflammatory response (e.g. Table 2).

Predictability of the art: The area of the invention is unpredictable. As discussed above, the method of *in vivo* gene therapy is highly complex and unpredictable. Indeed, recent gene therapy protocols have demonstrated unpredictable outcomes resulting from an unexpected inflammatory reaction to an adenoviral vector in a patient and the insertional mutagenesis of a gene resulting in a leukemia-like condition in children being treated for severe combined immunodeficiency (Edelstein et al, J. Gene Med. Vol. 6, pages 597-602, 2004, cited in a prior action; e.g. page 599, The hopes and the setbacks; Verma and Weitzman, pages 729-732, Clinical Trials: Successes and setbacks). The skilled artisan at the time the present invention was made recognized the difficulty of achieving sufficient heterologous gene expression to induce any therapeutic effect. Gene therapy is still a technique of the future and advancements in our understanding of the basics of gene delivery and expression must be made before gene therapy becomes a useful technique (e.g. Verma et al, p. 242, col. 2-3; Palù et al, pp. 10-11; Luo et al, p. 33, col. 1, 1st paragraph; Verma and Weitzman, page 732, 2nd full paragraph).

Furthermore, it would be unpredictable to use a stable mutant cell line to transiently inhibit homologous recombination. The working examples of the specification teach that the inhibition is constant characteristic of the cells.

Amount of experimentation necessary: The quantity of experimentation necessary to carry out the claimed invention is high, as the skilled artisan could not rely on the prior art or the present specification to teach how to make and use the claimed methods. With any nucleic acid one would have to determine how to deliver the given nucleic acid to the appropriate target cells with specificity and efficiency, and how to get sufficient expression to induce at least some therapeutic effect. Since neither the prior art nor the specification provides the answers to all of

these questions, it would require a large quantity of trial and error experimentation by the skilled artisan to do so.

In view of the breadth of the claims and the lack of guidance provided by the specification as well as the unpredictability of the art, the skilled artisan would have required an undue amount of experimentation to make and/or use the claimed invention. Therefore, claims 1, 2, 4, 5, 10-12 and 16-21 are not considered to be fully enabled by the instant specification.

Response to Arguments - 35 USC § 112

Applicant's arguments, see pages10-11, filed 5/24/2007, with respect to the rejection of claims 1, 2, 4, 5, 7, 11, 12 and 16-21 under 35 U.S.C. 112, second paragraph, have been fully considered and are persuasive. The previous rejection of claims 1, 2, 4, 5, 7, 11, 12 and 16-21 has been withdrawn.

The rejection of claim 7 under 35 U.S.C. 112, first paragraph, is moot in view of Applicant's cancellation of the claims in the reply filed 5/24/2007.

With respect to the rejection of claims 1, 2, 4, 5, 10-12 and 16-21 under 35 U.S.C. 112, first paragraph, Applicant's arguments filed 5/24/2007 have been fully considered but they are not persuasive.

The response asserts that *in vivo* embodiments refer to single cell organisms rather than multicellular organisms. This is not found persuasive, because the specification envisions practicing the claimed method *in vivo* in a multicellular organism, including a mammal, which encompasses humans (e.g., page 6 and paragraph bridging pages 11-12). The specification envisions the replacement of an inactive gene by an active gene, which is a type of gene therapy

(e.g., paragraph bridging pages 11-12). Accordingly, the claims read on or encompass a method of gene therapy. The claims do not need to be specifically drawn to in vivo methods to encompass those methods. In other words, the claims are not limited to *in vitro* embodiments or cultures of single cellular organisms.

The response asserts that the references provided on the IDS filed 1/25/2007 provide evidence that several species of *Aspergillus* and *Neurospora*, which are eukaryotic organisms that have a preference for non-homologous recombination, can integrate DNA by homologous recombination. Further, the response asserts that the five separate laboratories were able to perform the technique, which provides evidence that undue experimentation is not required. This is not found persuasive, because the evidence provided is not commensurate in scope with the claims. The claims are not limited to the method where the cell is an *Aspergillus* or *Neurospora* cell. The provided references do not provide evidence that experimentation required to practice gene therapy was routine in the art at the time the invention was made. In instant case gene therapy methods by homologous recombination are not considered routine in the art and without sufficient guidance to practice the method, the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. It is noted that the unpredictability of a particular area may alone provide reasonable doubt as to the accuracy of the broad statement made in support of enablement of claims. See *Ex parte Singh*, 17 USPQ2d 1714 (BPAI 1991). Therefore considering the state of the art and limited amount of guidance provided in the instant specification, one skill in the art would have to engage in excessive and undue amount of experimentation to exercise the invention as claimed.

For these reasons, and the reasons made of record in the previous office actions, the rejection is maintained.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1, 2, 5, 17-21 and 24 are rejected under 35 U.S.C. 102(b) as being anticipated by Liang et al (PNAS, Vol. 93, pages 8929-8933, 1996, cited in a prior action; see the entire reference). This rejection was made in the Office action mailed 10/20/2006 and has been extended to new claim 24.

Liang et al teach the claimed step of providing a mutant of Ku80, a component involved in nonhomologous recombination, in a CHO cell (*xrs-6* clones; e.g. Abstract; page 8929, paragraph bridging columns). Liang et al teach the introduction of a 3' neo sequence to correct a defect in a 5' neo sequence, by introducing a pCMV-I-SceI plasmid into an *xrs-6* clone (e.g. pages 8931-8932, Gene Targeting in CHO-K1 and *xrs-6* cells). The 3' neo sequence replaces a portion of the 5' neo sequence to replace an inactive gene and provide antibiotic resistance, where the antibiotic resistance is a desired property that allows the cell to survive as a result of the encoded therapeutic proteinaceous substance (e.g. pages 8931-8932, Gene Targeting in CHO-K1 and *xrs-6* cells; Figure 3). The neomycin nucleic acid sequence of interest is part of a

plasmid gene delivery vehicle (e.g. pages 8931-8932, Gene Targeting in CHO-K1 and *xrs-6* cells; page 8929, DNA Constructions).

Claims 1, 10, 16, 20, 21 and 24 are rejected under 35 U.S.C. 102(b) as being anticipated by Shiotani et al (Molecular and General Genetics, Vol. 248, pages 142-150, 1995; see the entire reference). This is a new rejection, necessitated by the amendment of claim 1 to recite the step of "providing said nucleic acid to said eukaryote" in the reply filed 5/24/2007.

Regarding claims 1 and 24, Shiotani et al teach that the filamentous fungus *Alternaria alternata* has a preference for nonhomologous recombination (e.g., page 248, right column). Shiotani et al teach a method of directing integration of a nucleic acid of interest to a predetermined site in the *Alternaria alternata* genome, comprising the steps of (i) linearizing a nucleic acid comprising a region homologous to the BRM1 gene of *Alternaria alternata*, and (ii) providing the nucleic acid to *Alternaria alternata* such that homologous recombination occurs (e.g., pages 145-146, Effect of double-strand breaks in transforming DNA on recombination; Figure 1; Table 1). Shiotani et al teach that the presence of a double strand break increases the percentage of transformants that undergo homologous recombination (e.g. Table 1). The step of linearizing the nucleic acid is a step of "steering an integration pathway towards homologous recombination."

Regarding claim 10, *Alternaria alternata* is a fungus (e.g., page 142, right column).

Regarding claim 16, Shiotani et al teach the method where the nucleic acid of interest comprises an inactive gene, which replaces the active BRM1 gene (e.g., page 143, left column, 1st full paragraph).

Regarding claim 20, the mutant BRM1 nucleic acid introduced into the *Alternaria alternata* confers the desired property of changing the color of the mycelia from dark green or black to light brown (e.g., page 143, left column, 1st full paragraph).

Regarding claim 21, Shiotani et al teach the introduction of the nucleic acid as part of a gene delivery vehicle (e.g., pages 145-146, Effect of double-strand breaks in transforming DNA on recombination; Figure 1).

Response to Arguments - 35 USC § 102

The rejection of claims 1, 2, 4, 10, 12 and 20 under 35 U.S.C. 102(b) as being anticipated by Jackson et al has been withdrawn in view of Applicant's amendment to the claims in the reply filed 5/24/2007. The claims have been amended to recite the step of "providing said nucleic acid to said eukaryote." Jackson et al do not explicitly teach the provision of a nucleic acid that has homology at or around a predetermined site.

With respect to the rejection of claims 1, 2, 5, 17-21 and 24 under 35 U.S.C. 102(b) as being anticipated by Liang et al, Applicant's arguments filed 5/24/2007 have been fully considered but they are not persuasive.

The response asserts that because targeted integration events are only obtained in the presence of the I-SceI endonuclease, Liang et al do not teach "steering an integration pathway towards homologous recombination." This is not found persuasive, because Liang et al specifically teach that the presence of the I-SceI endonuclease specifically steers the integration pathway towards homologous recombination. For example, Liang et al teach that a three to four order of magnitude stimulation of gene targeting by homologous recombination when the I-SceI

endonuclease is present (e.g., page 8932, left column, last paragraph; page 8931, left column, 1st full paragraph; Table 3). Thus, Liang et al teach "steering an integration pathway towards homologous recombination." Furthermore, Liang et al teach the method further comprising providing a mutant component involved in nonhomologous recombination, where the component is Ku80. Accordingly, the teachings of Liang et al meet each of the limitations of the rejected claims.

For these reasons, and the reasons made of record in the previous office actions, the rejection is maintained.

The rejection of claims 1, 2, 7 and 10 under 35 U.S.C. 102(b) as being anticipated by Tsukamoto et al has been withdrawn in view of Applicant's amendment to the claims in the reply filed 5/24/2007. The claims have been amended to recite the step of "providing said nucleic acid to said eukaryote." Tsukamoto et al do not teach providing a nucleic acid that has homologous at or around a predetermined site in a eukaryote with a preference for nonhomologous recombination. Tsukamoto et al teach recombination in *Saccharomyces cerevisae*, which has a preference for homologous recombination.

The rejection of claims 1, 2, 7 and 10 under 35 U.S.C. 102(b) as being anticipated by Moore et al has been withdrawn in view of Applicant's amendment to the claims in the reply filed 5/24/2007. The claims have been amended to recite the step of "providing said nucleic acid to said eukaryote." Moore et al do not teach providing a nucleic acid that has homologous at or around a predetermined site in a eukaryote with a preference for nonhomologous recombination. Moore et al teach recombination in *Saccharomyces cerevisae*, which has a preference for homologous recombination.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1, 5, 12, 16-21 and 24 are rejected under 35 U.S.C. 103(a) as being unpatentable over Jackson et al (WO 98/30902 A1, published July 16, 1998, cited in a prior action; see the entire reference) in view of Capecchi (Science, Vol. 244, No. 4910, pages 1288-1292, June 1989; see the entire reference). This is a new rejection, necessitated by the amendment of claim 1 to recite the step of "providing said nucleic acid to said eukaryote" in the reply filed 5/24/2007.

Jackson et al teach that Ku-associated DNA double-strand break repair (KDAR) can be inhibited to increase the efficiency of gene targeting and gene therapy (e.g. page 7, line 6; paragraph bridging pages 8-9). Jackson et al teach that two ways exist for repairing DNA double-strand breaks (DSBs): the first is through the process of illegitimate recombination (also known as DNA non-homologous end-joining or NHEJ), which is catalyzed by the KDAR system, and the second is the process of homologous recombination, whereby the damaged DNA molecule exchanges information with an undamaged DNA homologous partner DNA molecule (e.g. paragraph bridging pages 8-9). Jackson et al teach that the illegitimate pathway tends to predominate in mammalian cells, and inhibiting the KDAR system will make the proportion of DSBs repaired by homologous recombination increase and the efficiency of homologous gene targeting to a specific site will increase (e.g. paragraph bridging pages 8-9). Jackson et al teach

different method steps to accomplish the steering of recombination to the homologous recombination pathway: (1) inhibition of the interaction between XRCC4 and DNA ligase IV or XRCCR and DNA-Pkcs/Ku, which would be transient, and (2) providing a mutant of a component involved in KDAR (i.e. a non-homologous recombination system (e.g. page 10, lines 17-35; page 76, line 17 to page 77, line 17; page 78, line 30 to page 81, line 15). Jackson et al teach the step providing a mutant of Ku70 in a yeast strain and teach that Ku70 does not play a crucial role in the homologous recombination process (e.g. page 79, lines 12-24; page 81, lines 4-6). Jackson et al teach that Ku70 is present in both yeast and mammals.

Jackson et al do not specifically teach the provision of a nucleic acid of interest that has homology at or around a predetermined site. Jackson et al do not teach the replacement of inactive gene with an active gene, replacement of an active gene with an inactive gene, the introduction of a nucleic acid encoding a therapeutic proteinacious substance, the introduction of a nucleic acid that confers antibiotic resistance, the introduction or a nucleic acid confers a desired property, or the introduction of a nucleic acid in a gene delivery vehicle.

Capecchi teaches that gene-targeting technology can be applied to many types of human and mouse cells (e.g., page 1288). Capecchi teaches that the frequency of the replacement or insertion of sequence by homologous recombination into a predetermined site is dependent upon the extent of homology between the targeting vector and the endogenous DNA sequence (e.g., page 1289, right column, 1st full paragraph; Figures 2 and 3). Capecchi teaches that gene targeting can be used to replace an inactive gene with an active gene, or replace an active gene with an inactive gene, including the introduction of sequences that have a therapeutic property of conferring survival through antibiotic resistance (e.g., page 1289, right column, 2nd full

paragraph). Capecchi teaches that the nucleic acid is introduced in a gene delivery vehicle (e.g., Figure 2).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method of increasing the frequency of homologous recombination in a mammalian cell by inhibiting the KDAR system of Jackson et al to include the step of introducing a gene targeting vector comprising homologous sequence to a predetermined site taught by Capecchi because Jackson et al and Capecchi teach it is within the ordinary skill in the art to use homologous recombination for gene targeting.

One would have been motivated to make such a modification in order to receive the expected benefit of being able to make targeted modifications to the genome of a cell as taught by Capecchi (for example, creating an inactive gene in place of an active gene). Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 2 and 4 are rejected under 35 U.S.C. 103(a) as being unpatentable over Jackson et al (WO 98/30902 A1, published July 16, 1998, cited in a prior action; see the entire reference) in view of Capecchi (Science, Vol. 244, No. 4910, pages 1288-1292, June 1989; see the entire reference) as applied to claims 1, 16-21 and 24 above, and further in view of Gu et al (PNAS, USA, Vol. 94, pages 8076-8081, July 1997; see the entire reference). This is a new rejection, necessitated by the amendment of claim 1 to recite the step of "providing said nucleic acid to said eukaryote" in the reply filed 5/24/2007.

The combined teachings of Jackson et al and Capecchi are described above and applied as before.

Jackson et al and Capecchi do not specifically teach a mammalian cell with a mutation in Ku70, a component involved in nonhomologous recombination. Further, Capecchi et al teach that homologous recombination is performed in mouse embryo-derived cells as a vehicle to generate mice of any desired genotype.

Gu et al teach Ku70-deficient mouse embryonic stem cells (e.g., page 8078, paragraph bridging columns; Figure 1).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the combined teachings of Jackson et al and Capecchi to include the Ku70-deficient mouse embryonic stem cells taught by Gu et al because Jackson et al teach it is within the ordinary skill in the art to use a mammalian cell deficient in the KDAR pathway and the Ku70 deficient cells of Gu et al are deficient in the KDAR pathway. The cells of Gu et al are deficient in the KDAR pathway because they lack Ku70, which is required for nonspecific double-strand DNA end-binding activity and double strand break repair (e.g., Abstract; page 8078, right column, 2nd full paragraph).

One would have been motivated to make such a modification in order to receive the expected benefit of increasing the rate of homologous recombination, as taught by Jackson et al, in a cell type that is desirable for gene targeting experiments, as taught by Capecchi. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 1, 5, 11, 17, 20, 21 and 24 are rejected under 35 U.S.C. 103(a) as being unpatentable over Shalev et al (PNAS, USA, Vol. 96, pages 7398-7402, June 1999; see the entire reference) in view of De Block et al (The EMBO Journal, Vol. 3, No. 8, pages 1681-1689, 1984; see the entire reference).

Shalev et al teach a method of directing integration of a nucleic acid of interest to a predetermined site in a plant genome, where the nucleic acid has homology at and around said predetermined site, comprising the steps of (i) introducing a nucleic acid encoding RuvC into tobacco plants, and (ii) introducing plasmids pGS001 and pGS003, which have homology with each other at a predetermined site, into the RuvC-expressing plants through bolistic bombardment (e.g., page 7399, Tobacco Transformation and Bolistic Transformation and the ECR Assay). Shalev et al teach that the expression of RuvC results in a 11-fold increase in recombination between the plasmids (e.g., page 7399, left column, 1st paragraph). Thus, the expression of RuvC results in "steering an integration pathway towards homologous recombination." The result of the recombination reaction is the replacement of the inactive GUS with an active GUS, which confers the desirable property of conferring a color change to the cell (e.g., paragraph bridging pages 7399-7400). Furthermore, Shalev et al teach that plants have a preference for nonhomologous recombination (e.g., page 7398, right column, 1st full paragraph).

Shalev et al do note teach the delivery of the plasmid DNA with homology to the predetermined site by *Agrobacterium*.

De Block et al teach that *Agrobacterium tumefaciens* can be used to readily introduce DNA sequences into plant cells using the Ti plasmid vector pGV3850 and co-culture of

Agrobacterium containing the plasmid with the plant protoplasts (e.g., pages 1682-1683, Selection of transformed calli; page 1687, right column, 1st full paragraph; page 1688, plant cell culture methods).

Because both Shalev et al and De Block et al teach methods of introducing foreign DNA into plant cells, it would have been obvious to one skilled in the art at the time the invention was made to substitute the bolistic transformation of Shalev et al with the *Agrobacterium*-mediated transformation of De Block et al to achieve the predictable result of introducing the nucleic acid of interest into the plant cell in the method of Shalev et al.

Response to Arguments - 35 USC § 103

The rejection of claims 5, 11, 16 and 21 under 35 U.S.C. 103(a) as being unpatentable over Jackson et al in view of Bundock et al has been withdrawn in view of Applicant's amendment to the claims in the reply filed 5/24/2007. The claims have been amended to recite the step of "providing said nucleic acid to said eukaryote." The rejection was based upon introducing a nucleic acid into *Saccharomyces cerevisae*, which is not a eukaryote with a preference for nonhomologous recombination.

With respect to the application of the Jackson et al reference in the new rejections set forth above, Applicant's arguments filed 5/24/2007 have been fully considered but they are not persuasive.

At pages 13-14, the response asserts that the double strand break repair, exemplified in Jackson by repair of an extra-chromosomal plasmid, is not the same as integration of introduced DNA into the host chromosome. In response to applicant's argument that the references fail to

show certain features of applicant's invention, it is noted that the features upon which applicant relies (i.e., introduction of DNA into a host chromosome) are not recited in the rejected claim(s). Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims. See *In re Van Geuns*, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993). Furthermore, Jackson et al teach that the illegitimate pathway tends to predominate in mammalian cells, and inhibiting the KDAR system will make the proportion of DSBs repaired by homologous recombination increase and the efficiency of homologous gene targeting to a specific site will increase (e.g. paragraph bridging pages 8-9). Thus, the method of Jackson et al results in "steering an integration pathway towards homologous recombination." The teachings of Jackson directed to lig4 are not relied upon for the above rejections.

Conclusion

No claims are allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event,

however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jennifer Dunston whose telephone number is 571-272-2916. The examiner can normally be reached on M-F, 9 am to 5 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Joseph Woitach can be reached at 571-272-0739. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Jennifer Dunston, Ph.D.
Examiner
Art Unit 1636

/JD/

CELINE QIAN, PH.D.
PRIMARY EXAMINER